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Airway hyperresponsiveness (AHR) is a characteristic feature of asthma, and histamine and methacholine bronchoprovocation challenges have been widely used to document and quantitate AHR. In 1987, Pauwels et al proposed that stimuli used in bronchoprovocation could be divided into direct and indirect. The direct stimuli act directly on a specific airway smooth muscle receptor and include muscarinic agonists (ie, methacholine), histamine, leukotrienes, and prostaglandins. The indirect stimuli act through one or more intermediate pathways, many (but not all) of which involve release of mediators from inflammatory cells, primarily metachromatic cells. Mediator-releasing indirect stimuli include exercise, eucapnic voluntary hyperpnea, hypertonic saline, adenosine monophosphate (AMP), and mannitol. Indirect stimuli acting through other mechanisms include propranolol, tachykinins, and bradykinin, but these will not be further discussed in this article. Because the naturally occurring stimuli causing symptoms in asthma act through indirect mechanisms, Pauwels et al hypothesized that indirect airway responsiveness should correlate better with clinical features of asthma, including severity and control, current asthma activity, and so forth.

In this review, the direct challenges are considered. Because the methacholine inhalation challenge is both the most commonly performed and the only chemical challenge approved for human use in North America, it is the primary test considered.

**History and Methods**

In the 1940s, Robert Tiffeneau was the first to develop technology to measure maximal expiratory...
flow rates. He proposed that changes in expiratory flow rates following both bronchodilator (isoproterenol) and bronchoconstrictor (acetylcholine) administration could be valuable in assessing subjects with airway disease. Subjects with a 20% improvement in FEV$_1$ were considered to have a positive bronchodilator response. Likewise, subjects with a 20% fall in FEV$_1$ after the administration of a bronchoconstrictor were considered to have a positive test (ie, demonstrating AHR). Numerous different methods were developed for administration of histamine or methacholine. For safety purposes, challenges were started with a low dose, and the doses were increased until a response was achieved or an arbitrary maximum amount had been administered. No real attention was paid to performing a dose-response curve or to targeting an identifiable dose.

It is now appreciated that the provocative concentration causing a 20% fall in FEV$_1$ (PC$_{20}$) to a direct stimulus can be measured well into the normal range and that the measurement, namely the PC$_{20}$, is imprecise, with a best-case short-term repeatability of plus or minus one doubling dose or concentration. Consequently, there is an important need to standardize methods to achieve a reproducible dose of methacholine; the two major reasons are to identify normal from abnormal as clearly as possible with such an imprecise measurement and to compare values performed in different laboratories and by different methods.

Crapo et al have published details of two of the more commonly used methods. The 2-min tidal breathing method developed by Cockcroft et al involves inhalation of aerosol from a jet nebulizer, which is operated while continuously calibrated to an output of 0.13 mL/min. The dosimeter method modified from Chai et al involves inhalation of aerosol with five deep inspiratory capacity inhalations to a total lung capacity with a 5-s breathhold following each inhalation; the nebulizer is calibrated to deliver 9 µL per actuation. Except for the method of inhalation, the remainder of the methodologic recommendations are the same. Saline diluent is used as a baseline control and is then followed by doubling concentrations of methacholine from 0.03 mg/mL to 16 mg/mL or 32 mg/mL (or higher for research purposes). The inhalations are carried out with a 5-min interval between the start of one and the start of the next inhalation. Single measurements of FEV$_1$ are made at 30 s and 90 s after the completion of inhalation. The percentage decline in FEV$_1$ after methacholine is calculated from the FEV$_1$ following diluent. The test is completed when the top concentration is administered or the FEV$_1$ has fallen by 20%, and the PC$_{20}$ is calculated from the log concentration vs dose-response curve. For clinical purposes, the test can be shortened by adjusting the starting concentration based on the clinical picture, by omitting concentrations if there has been no response at a given concentration, or by using a quadrupling dose protocol.

Despite the difference in the volume of aerosol administered between methods, the two were believed to give similar results based on a single small study using histamine. It was proposed that the deep inhalation and breathhold probably resulted in greater retention of aerosol, better deposition of aerosol, or both; however, recent studies by two groups have shown that this is not the case. When the methods are performed as outlined by the American Thoracic Society, the tidal breathing method repeatedly produces a greater response (lower PC$_{20}$). There are two reasons: The first is the greater dose administered in the tidal breathing method, and the second, and likely more important, is that the dosimeter method, with its five maximal inspirations and breathholds, produces bronchodilatation and bronchoprotection in subjects specifically with mild AHR to methacholine. The results of the two methods are much more comparable when dealing with subjects with moderate or greater AHR (Fig 1). It is important because positive methacholine challenges in a routine diagnostic laboratory (tests done in subjects with symptoms that could be asthma but with normal resting lung function) most often will fall in the mild AHR range.

**Clinical Correlations**

The majority of the early work in the field of AHR was done with the direct challenges of histamine and methacholine. Histamine and methacholine produce very similar responses on a milligram-to-milligram or millimole-to-millimole basis and are, for simplicity here, considered identical. AHR, including variable airflow obstruction, is a constant and defining feature of the syndrome termed “asthma.” There is a correlation, with much overlap, between asthma severity and severity of AHR. Direct AHR increases with allergen exposure as demonstrated by Altounyan during the grass pollen season and as demonstrated by Cockcroft et al following allergen challenges in the laboratory. Direct AHR also improves with antiinflammatory therapeutic strategies, such as allergen avoidance and inhaled corticosteroids (ICS). Most studies have demonstrated a correlation between the severity of direct AHR and airway inflammation primarily with eosinophils or metachromatic cells; however, this correlation is only modest. Many groups have demonstrated that subjects with nonasthmatic airflow obstruction (ie, chronic airflow limitation) have increased response to direct stimuli that is closely related to the severity of the
Airway Hyperresponsiveness in Asthma

and the fixed component remains despite, at times, intensive treatment of asthma. It is now hypothesized that the variable component likely reflects changes in airway inflammation and that the fixed component likely reflects structural and functional changes in the airway that are persistent, if not permanent, and that have been labeled "airway remodeling." Because obstruction.\textsuperscript{24,25} This response is believed to be non-asthmatic in nature and likely represents a geometric issue with regard to airway diameter.

Consequently, 25 or 30 years ago a concept developed indicating that (direct) AHR had two components: variable and fixed. The variable component changes sometimes rapidly (eg, with allergen exposure, ICS), and the fixed component remains despite, at times, intensive treatment of asthma. It is now hypothesized that the variable component likely reflects changes in airway inflammation and that the fixed component likely reflects structural and functional changes in the airway that are persistent, if not permanent, and that have been labeled “airway remodeling.” Because

\textbf{Figure 1.} Comparison of two methacholine challenge methods in 55 subjects with asthma. The tidal breathing methacholine provocation concentration causing a 20% fall in FEV\textsubscript{1} (PC\textsubscript{20}) is on the left, and the dosimeter PC\textsubscript{20} performed with five total lung capacity inhalations of methacholine is on the right. The eight subjects noted in green have a positive methacholine PC\textsubscript{20} by tidal breathing (PC\textsubscript{20} \leq 8 mg/mL) and a negative challenge, dose corrected, for the dosimeter method with a PC\textsubscript{20} > 32 mg/mL. The five subjects in red have a dosimeter PC\textsubscript{20} between 16 mg/mL and 32 mg/mL, officially outside the normal range. Although there is a significant 1 doubling-dose difference in geometric means of PC\textsubscript{20}, the subjects represented in the lower half of the graph (PC\textsubscript{20} < 2 mg/mL) have similar intermethod values. (Reproduced with permission from Cockcroft and Davis.\textsuperscript{15})

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\caption{Comparison of two methacholine challenge methods in 55 subjects with asthma. The tidal breathing methacholine provocation concentration causing a 20% fall in FEV\textsubscript{1} (PC\textsubscript{20}) is on the left, and the dosimeter PC\textsubscript{20} performed with five total lung capacity inhalations of methacholine is on the right. The eight subjects noted in green have a positive methacholine PC\textsubscript{20} by tidal breathing (PC\textsubscript{20} \leq 8 mg/mL) and a negative challenge, dose corrected, for the dosimeter method with a PC\textsubscript{20} > 32 mg/mL. The five subjects in red have a dosimeter PC\textsubscript{20} between 16 mg/mL and 32 mg/mL, officially outside the normal range. Although there is a significant 1 doubling-dose difference in geometric means of PC\textsubscript{20}, the subjects represented in the lower half of the graph (PC\textsubscript{20} < 2 mg/mL) have similar intermethod values. (Reproduced with permission from Cockcroft and Davis.\textsuperscript{15})}
\end{figure}
Airway remodeling appears to relate to chronicity of disease, a function of both duration of disease and intensity of treatment, this fixed component is likely to be minor, if not absent, in subjects with recent-onset asthma. The transient nature of AHR is especially evident early on in occupational asthma when AHR can normalize within a few days of exposure. It is also almost certainly an issue in children with asthma who, in epidemiologic studies, frequently have normal histamine or methacholine challenges that likely are related to lack of clinically current exposures and symptoms.

The relationships outlined here are likely overly simplistic and have not been validated; therefore, they remain hypothetical. The relationship of the variable direct AHR to asthma activity, exposures, inflammation, and antiinflammatory treatments is indisputable; the mechanism, however, is uncertain. Although it has been attractive (and traditional) to attribute this transient AHR to (eosinophilic) inflammation either directly or indirectly (secondary to mucosal or smooth muscle effects), recent experience with some antiinflammatory therapies, particularly antiinterleukin-5 (mepolizumab), has dissociated AHR and eosinophils. The relationship of fixed AHR with structural changes is based on the (nonasthmatic) AHR seen in COPD and has not been fully validated in asthma. Nevertheless, it does appear, in my view, to relate to asthma duration and to at least a degree of residual airflow obstruction. Interestingly, I often have observed significant methacholine AHR in patients with mild or no symptoms with longstanding, sometimes previous, asthma, and this AHR does not seem to be associated with symptoms.

**Sensitivity and Specificity**

A major difficulty in assessing sensitivity and specificity of methacholine challenge relates to the difficulty in obtaining an independent objective confirmation of the presence of asthma (a so-called “gold standard”). In subjects with mild symptoms, often the best comparator is termed “doctor diagnosis.” Unfortunately, it is the lack of clinical assessment accuracy that requires the airway responsiveness testing in the first place, and therefore, discussions in this area become at times somewhat circular. Needless to say, the multiplicity of methods also poses a problem in assessing sensitivity and specificity.

The arbitrary cut points for the histamine and methacholine challenge have been defined to produce maximal sensitivity. Cockcroft et al initially chose 8 mg/mL, which identified all subjects with asthma. The test is also positive in a substantial portion of subjects with both allergic and nonallergic rhinitis and 4% to 5% of subjects without symptoms in a random population. This cut point of 8 mg/mL has now been expanded to a borderline range of 4 to 16 mg/mL (ie, 8 ± 1 mg/mL concentration). At 16 mg/mL, an even greater number of positive tests in subjects with no symptoms of asthma in a random population occurs; this may be as high as 20% from a review in a previously studied population. At a cut point or a 8 mg/mL or 16 mg/mL, using a non-deep inhalation method, the sensitivity and, consequently, negative predictive value for current symptomatic asthma approaches 100%. There are two caveats, the most important being that symptoms must be clinically current (ie, within the past few days); a negative methacholine challenge will not rule out seasonal asthma. The second important caveat, recently identified, is that for maximum diagnostic sensitivity, the methacholine should be inhaled by non-deep inhalation methods, which could involve either the tidal breathing method or a modified dosimeter method.

The specificity is not as good. Positive challenges may occur in many individuals with no asthma symptoms. The positive predictive value of a PC is <8 mg/mL in a random population is well below 50%, that is, <50% of the subjects in a random population with a PC <8 mg/mL have clinically current asthma symptoms. When the PC is <1 mg/mL (moderate AHR), the specificity and positive predictive value approach 100%.

Thus, the positive predictive value increases when the measured PC is lower. The positive predictive value is also higher in a group of individuals with a higher pretest probability (ie, those who are more likely to have asthma based on their symptoms). Although not validated, it is also thought that the positive predictive value will increase if the methacholine-induced symptoms resemble the naturally occurring symptoms for which the test was ordered.

**Direct and Indirect Comparisons**

The indirect challenges considered here will be limited to those involving inflammatory cell mediator release. Differences have been summarized recently (Table 1) and support the hypotheses outlined by Pauwels et al. Direct airway responsiveness measures airway smooth muscle function, whereas to a lesser extent, the variable component likely reflects airway inflammation. Airway caliber is important in determining response to direct stimuli. A relatively low dose is needed (approximate dose of methacholine inhaled at 16 mg/mL concentration is 1 mg). There is no limitation of the dose that can be administered, and the arbitrary cut points have defined a test that, with the caveats noted previously, is highly sensitive but not that specific. Minor tolerance or refractoriness occurs only at high doses and is limited to subjects...
The methacholine challenge is a highly sensitive direct challenge with a high negative predictive value. A negative methacholine challenge ($PC_{20} > 16 \text{mg/mL}$) excludes current asthma with reasonable certainty. The important caveats are that symptoms must indeed be quite recent, that methacholine be inhaled with no deep inhalations, and that both functional and specific antagonist medications must be withheld for the appropriate duration. There is the occasional elite athlete who has negative (deep inhalation method) methacholine challenges and positive exercise challenges; this is not the rule among regular individuals. A positive methacholine challenge in the moderate or greater range ($PC_{20} < 1 \text{mg/mL}$) has a high specificity and a high positive predictive value and at this level performs similarly diagnostically to the indirect challenges. The direct challenges will not identify individuals with corticosteroid-responsive airway disease not associated with variable airflow obstruction, the classic example being eosinophilic bronchitis (cough, eosinophilic inflammation, no AHR, and good response to ICS).

It is unlikely that indirect challenges would identify these individuals either. In this group of subjects, and indeed in individuals with asthma as a whole, a case could be made for studying sputum cell counts and differential in order to identify steroid responsiveness.

A methacholine $PC_{20}$ between 1 mg/mL and 16 mg/mL is consistent with but not diagnostic of asthma. Methacholine $PC_{20}$ values in this range are intermediate in sensitivity and specificity. The positive predictive value will increase as the $PC_{20}$ falls, the pretest probability for a diagnosis of asthma increases, and (probably) the methacholine-induced symptoms mimic the naturally occurring symptoms. Although not diagnostic of (clinically symptomatic) asthma, a $PC_{20}$ between 1 mg/mL and 16 mg/mL does provide a physiologic rationale for a trial of asthma therapy.

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